FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE BIODEGRADABLE FILM FROM YAM STARCH

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ABSTRACT

The purpose of this study is to produce biocomposite packaging film which is environmental friendly in nature and have potential to be used in the food packaging industry application. The objectives of this study are to fabricate different types of composite biodegradable film from yam starch and analyse antimicrobial activities of the films in resistance to *Escherichia coli* and *Bacillus subtilis*. Other than that, the films were characterized in term of physical and chemical properties. In this study, the biodegradable film will be fabricated with addition of antimicrobial agent to lead complete degradation process. Chitosan is used ad antimicrobial agent. The film preparation is by mix the yam flour with 1% acetic acid, chitosan, PEG 400 and water. Then, from the solution produced, the film is casted on the glass plate and left at ambient temperature until it has become dry. The film was then peeled from the glass plate after the drying is complete. The film is characterized by using fourier transform infrared (FT-IR) spectra, scanning electron microscope (SEM), antimicrobial activity, water solubility and viscosity. From the result, Fourier Transform Infrared (FT-IR) spectra analysis revealed starch crystallinity and hydrogen bonds were formed between chitosan and starch at wavelength 400-4000cm^{-1}. For water solubility, the film containing 3% chitosan only have 34.9578 % water solubility compared to film that containing 1% chitosan have 59.2309 % water solubility. Films containing 3% chitosan is also showed it has the highest viscosity value, which is 1413 cP at 20 rpm. From antimicrobial test, the result showed that, the more chitosan is used, the lower the colony to breed.

ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan filem pembungkusan biokomposit yang mesra alam dan mempunyai potensi untuk digunakan dalam aplikasi industri bungkusan makanan. Tujuan kajian ini adalah untuk menhasilkan komposit filem yang boleh terurai dari pati ubi serta menganalisa filem dalam merencat aktiviti mikrob Escherichia coli dan Bacillus subtilis. Selain itu, objektif kajian adalah untuk mengkaji filem dari segi fizikal dan kimia. Dalam kajian ini, filem boleh urai akan dibuat dengan penambahan agen antimikrob dalam meneruskan proses penguraian. Kitosan digunakan sebagai agen antimikrob. Penghasilan filem adalah dengan mencampurkan tepung ubi dengan asid asetik 1%, kitosan, PEG 400 dan air. Kemudian, dari larutan yang dihasilkan, filem ini dituang pada plat kaca dan dibiarkan pada suhu bilik sehingga kering. Filem ini kemudian dikupas dari plat kaca selepas proses pengeringan berlaku. Filem ini kemudian dikaji dengan menggunakan ‘Fourier Transform Infrared (FT-IR) spectra’, scanning electron microscope (SEM), ujian aktiviti antimikrob, kelarutan air dan kelikatan. Hasilnya, analisis spekta Fourier Transform Infrared (FT-IR) menunjukkan kanji terhablur dan ikatan hidrogen antara kitosan dan kanji terbentuk pada gelombang 400-4000 cm^{-1}. Untuk kelarutan dalam air, filem yang mengandungi kitosan 3% mempunyai kelarutan dalam air hanya 34.9578 % jika dibandingkan dengan filem yang mengandungi kitosan 1% yang mempunyai kelarutan dalam air 59.2309 % Filem yang mengandungi kitosan 3% juga menunjukkan nilai kelikatan tertinggi, iaitu 1413 cP pada 20 rpm. Dari ujian antimikrob, hasil menunjukkan lebih banyak kitosan digunakan, semakin kurang koloni yang dapat dibiakkan.

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LIST OF ABBREVIATIONS

FTIR	-	Fourier Transform Infrared
Mw	-	Weight Average Molecular Weight
PEG	-	Poly (ethylene glycol)
%v/v	-	volume percentage for chemical per basis
WS	-	Water Solubility
CS	-	Chitosan
E.coli	-	*Escherichia coli*
B.subtilis	-	*Bacillus subtilis*
TS	-	Tensile Strength
E	-	Elongation at break
SEM	-	Scanning Electron Microscopy

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Increased use of synthetic packaging films has led to serious ecological problems due to their total non-biodegradability. Continuous awareness by one and all towards environmental pollution by the latter and as a result the need for a safe, eco-friendly atmosphere has led to a paradigm shift on the use of biodegradable materials, especially from renewable agriculture feedstock and food processing industry (Tharanathan., 2003). Most widely used polymeric materials for packaging purposes, developed in the past 50–60 years, are durable and inert in the presence of microorganisms, leading to a long-term performance. However, in view of the current emphasis on environmental pollution problems and the shortage of land for solid waste management, the need for environmentally degradable polymers has increased (Mali *et al*., 2002). This development has received widespread government support in developing country. Several studies have been performed to analyze the properties of starch based biodegradable films.

Yams, the tubers of Dioscorea sp., are important staple food in many tropical countries. In Malaysia and Singapore, yam is also known 'taro'. Interestingly, yam is also been used as health food and herbal medicinal ingredients in traditional medicine (Hsu *et al*., 2003). In Japanese island, yams and yams products are regarded as a folk medicine for the treatment of importance, possibly because of the vegetable's high vitamin E content. Yam products generally have a lower glycemic index than potato products, which means that they will provide a more sustained form of energy, and give better protection against obesity and diabetes. Yam (Dioscorea sp) could be a good source of starch for the production of edible films and coatings, since its starch contains about 30% of amylose, and amylose is responsible for the film forming capacity of starches (Durango et al., 2006). Starch is one of the most commonly used raw materials to prepare biodegradable film, because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Shen *et al*., 2010).

Antimicrobial films and coatings have innovate the concept of active packaging and have been developed to reduce, inhibit or delay the growth of microorganisms on the surface of foods in contact with the packaged product (Durango et al., 2006). Although starch films have been widely study, but research on antimicrobial starch films is relatively rare and reported on yam. The addition of antimicrobial films or coatings can be more efficient than adding antimicrobial agents directly to the food. Antimicrobial films and coatings have innovate the concept of active packaging and have been developed to reduce, inhibit or delay the growth of microorganisms on the surface of foods in contact with the packaged product (Durango et al., 2006). 89% of outbreaks caused by food contamination by food workers, pathogens were transferred to food by workers' hands (Shen *et al*., 2010). Microbial contamination can controlled by antimicrobial substances incorporated into packaging materials by reducing the growth rate and maximum growth population and extending the lag-phase of the target microorganism or by inactivating microorganism by contact. Chitosan owning a broad spectrum of antimicrobial activity exhibits differing inhibitory efficiency against different fungi, Gram-positive and Gram-negative bacteria. Chitosan exerts an antifungi effect by suppressing sporulation and spore germination. In this study, the microorganism is used were *Escherichia coli* (E.*coli*) and *Basillus subtilis* (B.*subtilis*). This is because of to differentiate microbial activity based on Gram positive bacteria (B.*subtilis*) and Gram negative bacteria (E.*coli*). Furthermore, E.coli is the most common bacteria from human body, whereas B.*subtilis* is the most common bacteria that was found in the food.

Although there are many studies on petrochemical-based film that have been done, the demand for biodegradable polymer-based edible film and environmentally friendly has spurred interest, and chitosan is ideally positioned in this regard. Chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose] is a collective name for a group of partially and fully deacetylated chitin compounds due to its unique biological characteristics, including biodegradability and nontoxicity, many applications have been found either alone or blended with other natural polymers (starch, gelatin, alginates) in the food, pharmaceutical, textile, agriculture, water treatment and cosmetics industries (Kong *et al*., 2010).

The physical and biodegradable properties of a starch based biodegradable film are characterized by Fourier Transform Infrared (FT-IR) spectra, Tensile strength and elongation, Scanning Electron Microscopy (SEM). Then, to analyze the antimicrobial activity, the film will undergo antimicrobial test for antimicrobial analysis. The objectives of this study are to fabricate difference types of composite biodegradable films from yam starch, to evaluate antimicrobial activities of chitosan-incorporated yam starch films in resistance of *E.coli* and B.*subtilis* and to characterize the difference types of composite biodegradable film from yam starch.

1.2 Identification of Problem

Plastics are durable and their degradation process is very slow, possibly up to hundreds of year, the disposal of plastics contributes significantly to their environmental impact. With more and more plastics products, particularly plastics packaging, being disposed of soon after their purchase, the landfill spaced required by plastics waste in growing concern. The difficulties in collecting, identifying, transporting, cleaning and re-processing of plastics packaging materials often render the attempt of recycling non economical making disposal to landfill to more convenient alternative. The statistics showed over 67 millions tones of packaging waste are generated annually in certain developing country. In developed countries, food packaging represents 60% of all packaging (Davis and Song., 2006). Several studies have been performed to analyze the properties of starch based biodegradable films, but research on antimicrobial starch films is relatively rare.

1.3 Objectives

The main of this research are:

a) To fabricate difference types of composite biodegradable films from yam starch.
b) To evaluate antimicrobial activities of chitosan-incorporated yam starch films in resistance of *E.coli* and B.*subtilis*
c) To characterize the difference types of composite biodegradable film from yam starch.

1.4 Scope of Study

In general, the scopes of research are as following:

a) To study about the yam flour making process
b) To fabricate the composite biodegradable film from yam starch using fabrication process.
c) To study the physical properties of the film based on FT-IR, SEM and Tensile Strength (TS) & Elongation at break (E).
d) To study the antimicrobial activity of the films.

1.5 Significance of Study

The significance of this research are:

a) New alternative to replace nondegradable plastic.
b) Application in wide filed such as industry and medical
c) To save the environment
d) To reduce the landfill space.

CHAPTER 2

LITERATURE REVIEW

2.1 Yam

Yam (Dioscorea sp.) is widely cultivated in the tropical and subtropical regions of the world and is known for its high carbohydrate and medicinal value (Riley *et al*., 2008) In Taiwan, the fresh tuber slices are widely used as functional foods, and the dried slices are used as traditional Chinese herbal medicines. Yams (the tubers of the Dioscorea spp.), consumed and regarded as medicinal food in traditional Chinese herbal medicine, are seasonal foods and easily deteriorate during storage. It is of great importance to prolong the storage of yams for supplying in the off-season and without losing nutritional functionality (Hsu *et al*., 2003). The extracts of yam provide significant antioxidative activity and modified serum lipid levels in humans

Figure 2.1: Yam (Dioscorea sp.)

Yam (Dioscorea sp) could be a good source of starch for the production of edible films and coatings, since its starch contains about 30% of amylose, and amylose is responsible for the film forming capacity of starches (Durango *et al*., 2006).

On dry basis, the chemical composition of yam starch was: ash (0.17 ± 0.01%), protein (0.20 ± 0.01%), lipids (0.27 ± 0.02%) and starch (98.30 ± 0.05%). The amylase and amylopectin contents of native yam starch were 30 and 70%, respectively. This amylose content is relevant for the film forming capacity of the starch (Mali *et al*., 2002)

2.2 Starch

Starch is one of the major polysaccharides in plants and is in the form of granules that exist naturally within the plant cells (Mali *et al*., 2002) and is one of the most commonly used raw materials to prepare biodegradable film, because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Shen *et al*., 2010). Starch is a one of polysaccharide that have physical combination of branched and linear polymers (amylopectin and amylose, respectively), but it contains only a single type of carbohydrate, glucose.

Starch films possess low permeability and are thus attractive materials for food packaging. Starch is also useful for making agricultural mulch films because it degrades into harmless products when placed in contact with soil microorganisms (Chandra and Rustgi., 1998).

Starch based films and coatings exhibit different properties, attributed to the amylose content in the starch (Durango *et al*., 2006) and could contribute to new solutions in reducing the amount of plastic wastes and these polymers are obtained from renewable sources unlike synthetic polymers (Mali *et al*., 2004).

Starch is the main carbohydrate reserve in yam tubers, accounting for up to 85% of the dry weight matter. Yam starch occurs as granules, consisting of amylose (10 – 30%) and amylopectin (70 – 90%) molecules. (Riley *et al*., 2008) In Brazil, yam (Dioscorea alata) is being studied as an alternative source because of several desirable properties of its starch, such as, stability to high temperature and low pH.

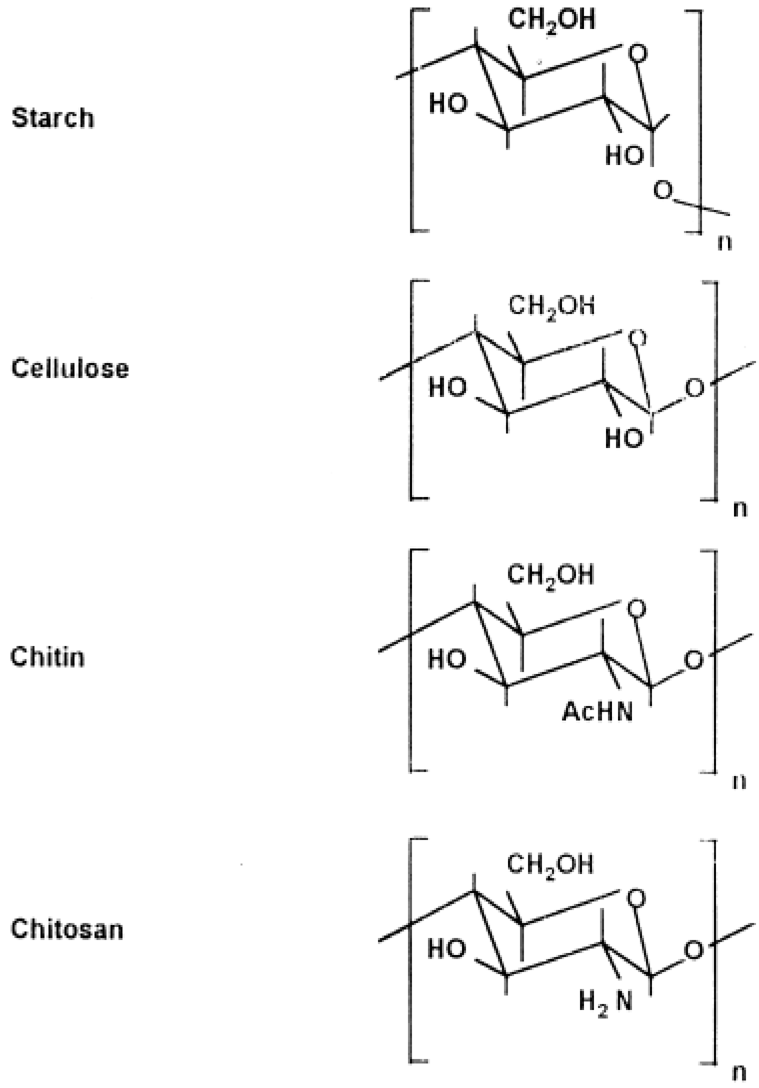

Figure 2.2: Structure of polysaccharides

Yam starch is a promising polymer for biofilm production due to their homogeneous matrix and stable structure at ambient conditions and relatively low water barrier properties (Mali *et al.*, 2004) and is widely study because it exhibits several desirable properties, such as stability at high temperatures and a low pH, high gelatinization temperature ranges (71.9 – 73.0–76.9 ^{o}C), high amylose contents (27 g/100 g), and restricted swelling (Fu *et al.*, 2005).

2.3 Chitosan

Chitin is consists of 2-acetamido-2-deoxy-b-D-glucose. Chitosan is the N-deacetylated derivate of chitin. Chitin and chitosan have a range of current and potential applications in photography, cosmetics, artificial skin, dressing, food and nutrition, ophthalmology, water engineering, metal capture from wastewater, paper finishing, solidstate batteries, drug delivery system, biotechnology, cell-stimulating materials (Shih *et al*.,2009) pharmaceutical, textile, agriculture, water treatment and cosmetics industries (Kong *et al*., 2010). The table below is showed the application of chitosan.

Table 2.3: Table of chitosan application

Application of antimicrobial property of chitosan.

Support (preparation method)	Application	Tested microorganism
Chitosan acetates	Food preservative[a]	*Escherichia coli* *Staphylococcus aureus*
Chitosan and its Maillard reaction products	Food preservative[a]	*Bacillus subtilis* CCRC 10258
Chitosan-hydroxy propyl methyl cellulose film	Packaging materials[a]	*Listeria monocytogenes*
Chitosan/polyethylene oxide film	Packaging materials[a]	*Escherichia coli*
Chitosan-nylon-6/Ag blended membranes	Packaging materials[a]	*Escherichia coli* *Staphylococcus aureus*
Polypropylene/chitosan/pectin films	Packaging materials[a]	Bacteria: *Clavibacter michiganensis* *Pseudomonas solanacearum* Fungi: *Fusarium oxysporum* *Verticillium albo-atrum* *Alternaria solani*
Chitosan-hydroxy propyl methyl cellulose film	Edible films and coatings[a]	*Aspergillus niger*
Chitosan	Food additive[a]	*Streptococcus*
Alginate/chitosan fibers	Wound dressing materials[b]	*Staphylococcus aureus*
Quaternised chitosan nano-fibers	Wound-healing applications[b]	*Escherichia coli* 3588 *Staphylococcus aureus* 749
Quaternized chitosan derivative/poly (vinyl pyrrolidone) fibers	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Alginate/carboxymethyl chitosan blend fibers	Wound dressing materials[b]	*Staphylococcus aureus*
Polypropylene-g-acrylic acid-g-N-isopropylacrylamide-chitosan fabric	Wound dressing materials[b]	*Pseudomonas aeruginosa* *Staphylococcus aureus*
Chitosan/cellulose blends membrane	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Chitosan-$Ca_3V_{10}O_{28}$ complex membrane	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Porous chitosan/poly(N-isopropylacrylamide) gel/polypropylene sponge	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Chitosan-gelatin sponge	Wound dressing materials[b]	*Escherichia coli* K88 *Streptococcus*
Photocrosslinkable chitosan hydrogel	Wound dressing and tissue adhesion[b]	*Escherichia coli*
Poly(vinyl alcohol)/water-soluble-chitosan hydrogels	Wound dressing materials[b]	*Escherichia coli*
Chitosan/poly(vinyl alcohol) blended hydrogel membranes	Haemodialysis[b]	*Aurococcus*

The ideal antimicrobial polymer should possess the following characteristics: (1) easily and inexpensively synthesized, (2) stable in long-term usage and storage at the temperature of its intended application, (3) not soluble in water for a water-disinfection application, (4) does not decompose to and/or emit toxic products, (5) should not be toxic or irritating to those who are handling it, (6) can be regenerated upon loss of activity, and (7) biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact (Kong *et al*., 2010).

Chitosan films are biodegradable, biocompatible, flexible, durable, strong, tough and hard to break, have moderate values of water and oxygen permeability, decrease the respiratory rate of food and also inhibit the microbial growth (Agullo *et al*., 2003). Chitosan obtained from chitin (of shrimp waste silage) has low molecular weight compared to the commercial chitosan. The low molecular weight of chitosan obtained can be attributed to greater susceptibility to degradation of chitin and/or depolymerization during the removal of proteins and minerals in the silage, and in the purification procedures and subsequent deacetylation (Camacho *et al*., 2010).

Due to its property of inhibiting the growth of many pathogenic bacteria and fungi, chitosan has widely been used in antimicrobial films and coatings. In some fungi, by interaction with the strongly electronegative microbial surface leading to changes in permeability, metabolic disturbances, and eventually death, chitosan can produce alterations of membrane functions. Chitosan antimicrobial activity against bacteria could be due to the polycationic nature of its molecule, which allows interaction and formation of polyelectrolyte complexes with acid polymers produced at the bacteria cell surface (lipopolysaccharides, teichoic and teichuronic acids or capsular polysaccharides (Durango *et al*., 2006).

Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts. Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria, however, it is low toxicity toward mammalian cells. In many food applications, low water solubility forces chitosan to be dissolved in dilute acid solution, such as acetic or lactic acid solution. This acidic ambience can adversely affect chitosan molecules via hydrolysis and chain depolymerization. The development of modified water-soluble derivative is an efficient approach in practice and the chitosan structure is chemically more stable compared with the acidic solution. It was observed that, chitosan acetate inhibited the growth of two main waterborne food pathogens, E. *coli* and S. *aureus* (Kong *et al*., 2010).

Most of the mechanical properties of chitosan films are comparable to those of commercial polymers of medium strength such as cellulose. The mechanic and permeable properties of chitosan films can be controlled by selecting molecular weight, a suitable solvent system and the addition of plasticizer agents, dispersants and compatibilizers, among however, the presence of such compounds can affect the antimicrobial activity of chitosan films (Camacho *et al*., 2010).

2.4 Petroleum-Based Film

The majority of plastic products are made from petroleum-based synthetic polymers that do not degrade in a landfill or in compost like environment. High molecular weight synthetic polymers containing largely C-C bond are generally resistant to biodegradation because microbial enzymes are not accessible to them due to their hydrophobic nature (Muthukumar *et al*., 2010).

2.5 Biodegradable Film

Biodegradable is a polymer blends and composites containing natural polymers as biodegradable additives (such as starch, cellulose and their derivatives) (Alain and Sylvie., 2008)

Biopolymer films and coatings from polysaccharides, proteins and lipids, formulated either with one or more components have the potential to control mass transfer and thus extend food shelf life and this study is also shows starch films are excellent oxygen barriers, due to their tightly packed, ordered hydrogen-bonded network structure and low solubility (Mali *et al*., 2004).

Film based food preservative materials are safer and more widely applied presently, especially as food package. Various kinds of chitosan-based packaging films modified with new polymeric material have been developed. The process endows these cooperating films with antimicrobial property as well as advantageous mechanical characteristics as showed in table 2.3. Beside of the biodegradability, environmental attribute is an important functional attribute. Thus, the concept of biodegradability enjoys both user-friendly and eco-friendly attributes, and the raw materials are essentially derived

Biodegradable polymer films are not meant to totally replace synthetic packaging films, but to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function. For instance, biodegradable and edible films can be used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to the commercial packaging materials (Bourtoom and Chinnan., 2008).

An additional advantage of biodegradable packaging materials is that on biodegradation or disintegration and composting they may act as fertilizer and soil conditioner, facilitating better yield of the crops (Tharanathan., 2003)

2.5 Plasticizer

The addition of plasticizers overcomes starch film brittleness and improves flexibility and extensibility. Plasticizers must be compatible with the film forming polymer. They reduce intermolecular forces and increase the mobility of polymer chains. Hydrophilic compounds such as polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers in hydrophilic film formulations (Mali *et al*., 2004). The mechanical properties of chitosan film were improved by blending with PEG oligomer as plasticizer (Suyatma et al., 2005). Hydrophilic compounds such as polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers in hydrophilic film formulations and avoid cracking of the film during handling and storage and affect gas, water vapor and solute permeabilities (Mali *et al*., 2004).

Poly(ethyleneglycol) (PEG) is fascinating synthetic polymer. It shows biodegradability, biocompatibility, less toxicity and hydrophilicity and has been used in many kinds of applications (Roberts *et al*., 2002). The more the content of PEG increased in the film forming solution, the more the water solubility decreased (Sebastien *et al*., 2006). PEG concentration also influent to the flexibleity of the film. The higher concentration PEG was used, the more flexible they were.

When incorporated into human body, PEG with molecular weight lower than 20 kDa but higher than 400 Da is cleared immediately without structural change in the urea.Whereas lowmolecular-weight oligomers of PEG of about 400 Da or less is degraded in vivo by alcohol dehydrogenase to toxic metabolites, but PEG with molecular weight above 1000 Da is safety, and so, non-toxic (Tanuma et al., 2010).

2.6 Film characterization

2.6.1 Tensile strength and Elongation at break

Encountered during its application and subsequent shipping or handling of the food, biodegradable film must endure the normal stress to maintain its integrity and barrier properties. Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break, respectively. (Bourtoom and Chinnan., 2008),

Elongation at the break (E) is defined as an indication of the films' flexibility and stretch ability (extensibility). Elongation of film is determined at the point when the films breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of a films to stretch.

2.6.2 Water Solubility

Water resistance is an important property of biodegradable or edible films for applications as food protection where water activity is high, or when the film must be in contact with water during processing of the coated food (e.g. to avoid exudation of fresh or frozen products) (Bourtoom and Chinnan., 2008).

Normally, higher solubility would indicate lower water resistance, but in some case, high solubility may be an advantage for some applications such as in situations when the films will be consumed with a product that is heated prior to consumption and may also be an important factor that determines biodegradability of films when used as packaging wrap.

2.6.3 Fourier Transform Infrared (FT-IR) spectra

FTIR spectroscopy was used to determine the interactions between yam starch and chitosan (Bourtoom and Chinnan., 2008). When two components are mixed, the physical blends versus chemical interactions are affected by changes in the characteristic spectra peaks (Shen *et al*., 2010).

2.6.4 Degradation factor

There are three environment effects on the degradation of starch and pro-oxidant blended polyolefins film, which are marine environment, soil burial and open sunlight. Marine environment is found to be one of the major sites for dumping of waste especially plastics, and there is a need to develop strategies to degrade them faster than what is observed from current studies. Burying plastic in soil is not a good idea since it may persist longer than the other two strategies. Open sunlight appears to be a good way to subject polyolefins to abiotic degradation/deterioration (Muthukumar *et al., 2010*).

2.6.5 Soil Burial

Aerobic soil environments generally contain consortia of several different types of degrading bacteria and fungi which operate cooperatively. This is the process of degradation cellulose to glucose and cellodextrins by primary microorganisms, and secondary microorganism. The final products from aerobic biodegradation are ultimately CO2 and water. Increase in carbonyl index at the end of 150 days in sample exposed to UV and soil clearly reveal that the oxidation has taken place (Muthukumar *et al., 2010*).

Bacteria present in soil are important agents for material degradation. Particularly affected are cellulosic plant life, wood products, and textiles subject to cellulytic degradation Chandra and Rustgi., 1998).

Starch is normally the main site for biodegradative attack in starch-containing biocomposites.(Vilaplana *et al*., 2010)

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, the mothod that used in this study is described extensively. In general, the method can be divided into two phases. The first phase is to fabricate film from yam starch, and the second phase is to characterize the film.

3.2 Materials

Yam was obtained from a local farmer in Kuantan. All chemicals like acetic acid glacial, polyethylene glycol 400, Chitosan is provided from University Malaysia Pahang (UMP) laboratory. E.*coli* and B.*subtilis* were cultured and maintained in the microbiology laboratory of UMP.

3.3 Equipments and Apparatus

Apparatus that involved in this study are domestic blender, sieve (shaker), mortar and pestle, polyethylene bags, glass plates, biker, stirrer and petri dish. Meanwhile for the equipment, the film is characterized by using fourie transform infrared, EVO 50 EDX scanning electron microscope, tensile INSTRON Instrument and viscosity meter.

3.4 Film Fabrication

3.4.1 Production of Yam Flour and Starch Isolation

Yam tubers were rinsed, peeled, diced and then, disintegrated in a domestic blender with 100 ml distilled water. The mixture was sieved with filter cloth and the solids retained were exhaustively rinsed on the sieve with the distilled water again. The filtrate was allowed to stand at room temperature for overnight and decant was discarded. Starch precipitate will be produced at the bottom of the solution that was filtered overnight. Solution at the top is removed while the starch precipitate at the bottom will be filtered by using filter paper. The filtered starch will leave under room temperature for 24 hours. Then, on the next day, the filtered starch will be dried in a convection oven at 40 C until 12% moisture. Lastly, the starch was ground with a mortar and pestle to pass a 0.105 mm sieve and then stored in powder jar.

Figure 3.4.1: Yam starch

3.4.2 Solution Preparation and Film Fabrication

4g of starch was dispersed in 100ml distilled water was stirred for 1 hour at room temperature, and heated to 100^{o}C. After gelatinization, 5ml polyethylene glycol 400 was added as a plasticizer and the resulting dispersion was subjected to further mixing for 30 minutes. To prepare for the second solution, which is chitosan solution, chitosan was mixed at different concentration (1%, 2% and 3%) in the 100ml 1% acetic acid aqueous solution. after that mixed both of solution together for 1 hour. To prepare the antimicrobial film, the solution that produced will degassed under vacuum for 1 hour. Next, the warm mixture was casted on the framed glass plates and left at ambient temperature until it has become dry. The film was then peeled off the glass plate after the drying is complete.